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COMPOSITION OF CITRUS LEAVES AT VARIOUS STAGES OF MOTTLING

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A study of the soil factors influencing the mottling of Citrus leaves in southern California showed that the percentage of humus in the soils of the Citrus groves varied inversely with the mottling of the leaves, and this inverse relation showed a correlation of 67 per cent (2).¹ In another study it was found that organic matter, whether derived from stable manures, artificially produced manures from various green cover-crop materials, or by acid hydrolysis of sugar and hay material, attacked the soil minerals and liberated iron, calcium, magnesium, and phosphoric acid.² In another study on the mulched-basin system (3) it was found that on certain soil types this system caused a considerable diminution of leaf-mottling on orange trees (*Citrus aurantium*).

In view of these three facts it seemed possible that the mottling of Citrus leaves in southern California might possibly be due in part to a lack of supply of the mineral nutrients supposed to be most closely connected with the formation of chlorophyll. For this reason, Citrus leaves in various stages of mottling were collected and the iron, magnesium, calcium, and phosphoric-acid contents determined. The leaves in the various stages of mottling were collected from trees in the same grove, and in some cases from the same trees, so as to eliminate, as far as possible, the influence of variations in soil type.

Willstätter (11) has established the fact that the chlorophyll of all classes of plants contains magnesium and no other metal, and that the magnesium in the chlorophyll has an important part in the assimilation of carbon dioxide. Mameli (5, 6), in growing a number of plants in media lacking in magnesium, found that the plants were etiolated or pale green and the chloroplasts abnormal in form as well as in color. She further states that comparative analysis showed a smaller percentage of magnesium in chlorotic or discolored leaves or leaf parts than in normal portions of the same plant.

¹ Reference is made by number to "Literature cited," p. 166.

² Jensen, Charles A. Effect of decomposing organic matter on the solubility of certain inorganic constituents of the soil, particularly in its bearing on mottle-leaf of Citrus trees. *Not yet published.*

In another place (4) the same author reports that plants growing in media lacking manganese remained colorless or very pale and that by adding manganese in increasing proportions a corresponding increase in green color resulted.

Stoklasa, Brdlik, and Just (10), in repeating former work, reaffirm their earlier conclusion that phosphorus plays an important rôle in the production of chlorophyll. Their method of procedure was to extract the leaves with benzol and to determine the phosphorus in the extract.

One of the authors above cited, Brdlik (1), states that the physiological action in plants indicates a close relation between phosphorus and chlorophyll in the plant cell. He also states that alcohol and benzol extracts of green leaves show inorganic phosphorus as well as colorless phosphates, indicating that phosphorus plays a very important rôle in chlorophyll formation. It is his opinion that phosphorus plays as important a part in the physiological activity of the plant as magnesium or potassium.

Schryver (9) states that chlorophyll is a magnesium derivative from which the metal is eliminated readily by acids, but with great difficulty by alkalis.

Palladine (8) studied the influence of various carbohydrates on chlorophyll formation. Etiolated plants of *Vicia faba* and *Phaseolus vulgaris*, after being kept in the dark for 48 hours, were brought into the light. He states that the following substances favored the formation of chlorophyll: Saccharose, raffinose, glucose, fructose, maltose, glycerin, galactose, lactose, and dextrin. The following substances checked the formation of chlorophyll: Mannite, dulcitol, asparagin, urea, alcohol, ammonium chlorid, and quinic acid.

Mazé, Ruot, and Lemoigne (7) state that they produced chlorotic conditions in *Vicia narbonensis* by adding 0.2 per cent of calcium carbonate to the culture medium, which condition was neutralized in three days by applying a few drops of iron nitrate to the leaves.

There are apparent contradictions in the findings of some of these authors. Willstätter states (11) that the chlorophyll of all plants contains magnesium and no other metal; Mameli (5, 6) that plants became chlorotic in the absence of manganese; Stoklasa et al. (10) that phosphorus plays an important part in the formation of chlorophyll.

It may be that the confusion on the subject is increased by mistaking an indirect influence of some of the above-mentioned elements for a direct one. For instance, iron was for a long time considered an essential constituent of chlorophyll, and, while it was later found not to be the case, iron is known to be quite essential in the formation of the green pigment in plants.

Palladine's results (8) on chlorophyll formation as influenced by various carbohydrates may be of great practical importance in orcharding. Especially may this be so in a system under so thorough control as the

mulched basin, when the decomposition products come into direct contact with the rootlets of the tree.

The mineral composition of Citrus leaves is subject to much variation, and the age of the leaf is an important factor to bear in mind when selecting leaves for comparative study. Since Citrus trees retain their leaves for two to three years, it is often difficult to determine the age of a certain leaf. Especially is this true of a leaf in the last stages of mottling, which, even when but 2 or 3 months old, often looks to be 2 or 3 years old.

It is also not a safe procedure to compare mottled leaves from a grove in a badly mottled condition with healthy leaves from another grove in a good condition, especially if the two groves are on different soil types.

In describing the mottled conditions of the leaves here discussed, the terms "first stage," "second stage," and soon to the "fifth stage" were used. These terms mean that stage 1 represents the first definite appearance of mottling, the mottled or chlorotic spots being limited to one to three small spots on each side of the midrib, the spots in this stage being usually confined to the upper part of the leaf. The fifth, or last, stage is the other extreme, which represents a leaf where only the midrib may retain a little chlorophyll or none at all. The stages 2, 3, and 4 simply represent conditions of increasing mottling and range between stages 1 and 5. The various stages are represented in color in one of the publications already cited (2).

In Table I are given the percentages of iron, calcium, magnesium, and phosphoric acid found in orange and lemon leaves in various stages of mottling, and also the relative distribution of these elements. The leaves were all of new spring growth and were collected on the fertilizer experiment plots of the University of California Citrus Experiment Station, Riverside. In each table the healthy leaves—that is, those with only a trace of mottling—are from trees on the manure plots, and the mottled leaves are from trees on the sodium-nitrate plots. These plots are side by side and represent very extreme tree conditions not only so far as leaf mottling is concerned but also as regards foliage density, fruiting, etc.

Considering the average composition of the entire leaves, the percentages of calcium, magnesium, and phosphoric acid increase as the mottling increases, in both the orange and lemon leaves. There is but one exception, that of the phosphoric acid in the last stage of mottling in the lemon leaves.

In most cases the ratio of the percentages of the determined elements in the leaves to those in the midribs diminishes slightly as mottling increases, though this variation is not very marked.

In each kind of leaves the average percentage of iron is highest in the healthy leaves and is least in stages 3 and 4.

TABLE I.—Composition of orange and lemon leaves in various states of mottling. Leaves collected on May 11, 1916

ORANGE LEAVES

Part analyzed.	Stage of mottling.	Percentage distribution.				Percentage on dry substance.			
		Fe.	Ca.	Mg.	P ₂ O ₅ .	Fe.	Ca.	Mg.	P ₂ O ₅ .
Leaves minus mid-ribs.	Trace....	44	40	44	35	0.0096	2.17	0.266	0.437
Midribs alone.....	do.....	44	36	27	31	.0092	1.98	.162	.395
Leaf stems.....	do.....	10	24	29	34	.0020	1.35	.171	.433
Average of leaves and midribs.....						.0094	2.08	.214	.417
Leaves minus mid-ribs.	3-4	Tr.	34	40	38	Tr.	2.18	.300	.517
Midribs alone.....	3-4	45	39	35	30	.0022	2.52	.262	.411
Leaf stems.....	3-4	54	27	25	32	.0027	1.71	.190	.446
Average of leaves and midribs.....						.0011	2.35	.281	.464
Leaves minus mid-ribs.	Last....	62	31	39	35	.0058	3.26	.342	.526
Midribs alone.....	do.....	17	41	37	31	.0016	4.27	.324	.458
Leaf stems.....	do.....	21	28	24	34	.0017	2.96	.202	.513
Average of leaves and midribs.....						.0037	3.77	.333	.492

LEMON LEAVES

Leaves minus mid-ribs.	Trace....	45	41	38	37	0.0160	1.75	0.216	0.454
Midribs alone.....	do.....	45	35	31	31	.0160	1.49	.173	.386
Leaf stems.....	do.....	10	24	31	32	.0036	1.05	.175	.400
Average of leaves and midribs.....						.0160	1.62	.200	.420
Leaves minus mid-ribs.	3-4	52	44	33	38	.0120	2.26	.226	.657
Midribs alone.....	3-4	34	37	38	31	.0080	1.91	.259	.543
Leaf stems.....	3-4	14	19	29	31	.0033	0.98	.196	.535
Average of leaves and midribs.....						.0100	2.09	.243	.600
Leaves minus mid-ribs.	Last....	30	29	40	22	.0132	3.42	.337	.243
Midribs alone.....	do.....	42	39	40	33	.0180	4.68	.336	.370
Leaf stems.....	do.....	28	32	20	45	.0120	3.78	.166	.513
Average of leaves and midribs.....						.0156	4.00	.387	.309

In the healthy leaves and usually also in the less mottled leaves of both orange and lemon the percentage of each mineral element is less in the midrib than in the mesophyll tissue. In leaves in the last stage of mottling, however, there is no definite uniformity in this respect, the midrib containing in some cases a higher and in some cases a lower percentage than the leaf proper. There does not appear to have been any difficulty about the transfer of these mineral elements from the conducting tissue to the mesophyll areas.

In most cases, regardless of the stage of mottling, the leaf stems contain less iron, calcium, and magnesium than either the midrib or mesophyll. The phosphoric acid is more evenly distributed in the three portions of the leaf.

Table II shows the results of analyses of orange leaves collected from a commercial grove near Riverside. There was decidedly less calcium and a little less magnesium in the leaves in the medium stage of mottling than in the healthy leaves or in the worst mottled leaves; otherwise, the remarks regarding the leaves discussed from Table I apply to those given in Table II.

TABLE II.—Composition of orange leaves in various stages of mottling. Leaves collected on April 13, 1916

Part analyzed.	Stage of mottling.	Percentage distribution.				Percentage on dry substance.			
		Fe.	Ca.	Mg.	P ₂ O ₅ .	Fe.	Ca.	Mg.	P ₂ O ₅ .
Leaves minus midribs.	None . . .	39	44	40	39	0.0126	2.88	0.261	0.436
Midribs alone.	do . . .	39	39	33	33	.0126	2.35	.214	.368
Leaf stems.	do . . .	22	20	27	28	.0075	1.35	.173	.308
Average of leaves and midribs.						.0126	2.62	.238	.402
Leaves minus midribs.	2-3 . . .	48	40	41	38	.0250	1.96	.242	.455
Midribs alone.	2-3 . . .	32	35	31	32	.0167	1.73	.183	.376
Leaf stems.	2-3 . . .	20	25	28	30	.0105	1.27	.159	.300
Average of leaves and midribs.						.0209	1.85	.213	.416
Leaves minus midribs.	3-4-5 . . .	26	34	37	31	.0185	3.22	.294	.400
Midribs alone.	3-4-5 . . .	29	42	36	35	.0200	4.05	.285	.450
Leaf stems.	3-4-5 . . .	45	24	27	34	.0316	2.28	.220	.430
Average of leaves and midribs.						.0193	3.64	.290	.425

The leaves reported in Table III were collected on January 3, 1916, in commercial groves near Riverside. These leaves, except the last set given, were collected from the same grove; usually the healthy and mottled leaves were taken from the same tree branches, where both

healthy and mottled leaves of approximately the same age could be obtained.

As in the preceding determinations, the worst mottled leaves have the highest percentage of the mineral elements under investigation; and the percentage distribution shows no consistent differences between the conducting tissue and the mesophyll areas.

TABLE III.—*Analysis of orange leaves in various stages of mottling. Leaves collected on January 3, 1916*

Part analyzed.	Stage of mottling.	Percentage distribution.			Percentage on dry substance.		
		Ca.	Mg.	P ₂ O ₅ .	Ca.	Mg.	P ₂ O ₅ .
Leaf minus midrib.....	None.....	37	34	32	2.87	0.332	0.500
Midribs alone.....	do.....	39	39	35	3.07	.491	.482
Leaf stems.....	do.....	24	29	29	1.85	.294	.400
Average of leaves and midribs.....					2.97	.367	.491
Leaf minus midrib.....	2-4.....	50	44	49	2.28	.275	.531
Midribs alone.....	2-4.....	50	56	51	2.30	.323	.551
Average of leaves and midribs.....					2.29	.299	.541
Leaf minus midrib.....	4-5.....	43	51	40	1.78	.267	.563
Midribs alone.....	4-5.....	57	49	60	2.39	.261	.855
Average of leaves and midribs.....					2.09	.264	.699
Leaf minus midrib.....	Last (leaves all yellow, midribs green).....	30	37	32	2.66	.344	.530
Midribs alone.....	do.....	41	32	39	3.67	.298	.646
Leaf stems.....	do.....	29	31	29	2.62	.294	.473
Average of leaves and midribs.....					3.17	.321	.588
Leaves.....	Very spotted; yellow spots.....	28	27	23	2.69	.204	.493
Do.....	Green spots; mostly veins.....	34	42	31	3.24	.319	.553
Midribs.....	do.....	38	31	46	3.68	.242	.796
Average of leaves and midribs.....					2.97	.262	.483

The last set of leaves given in Table III was collected in another grove, where the mottled appearance of the leaves was much accentuated, the boundary lines between the chlorotic tissue and the green tissue being very sharp. The mottled spots were cut out and these and the remaining green parts, mostly veins, were separately analyzed. The green areas contain decidedly more of the elements determined than the yellow spots,

but there does not seem to have been any definite accumulation of these elements in the midribs, with the exception of phosphoric acid. The average percentage of the various elements in the whole leaf is about the same as in the other leaves reported in Table III.

Table IV shows a comparison of the composition of old and new leaves of grapefruit (*Citrus decumana*). The difference in the calcium and the magnesium contents is striking, though the amount of phosphoric acid is about the same in the old and new leaves. The results show the importance in a study of this kind of securing leaves of as nearly the same stage of development as possible.

TABLE IV.—Analysis of old and new grapefruit leaves. Collected on January 3, 1916

Part analyzed.	Description.	Percentage distribution.			Percentage on dry substance.		
		Ca.	Mg.	P ₂ O ₅	Ca.	Mg.	P ₂ O ₅
Leaves minus midribs...	Old leaves under the new leaves given below.	49	48	52	5.06	0.425	0.455
Midribs alone.....	do.....	51	52	48	5.24	.471	.416
Average.....					5.15	.448	.436
Leaves minus midribs...	New leaves above the old leaves given above.	47	56	57	2.13	.332	.560
Midribs alone.....	do.....	53	44	43	2.41	.260	.431
Average.....					2.27	.296	.496

Table V shows the analyses of leaves from a privet plant (*Ligustrum aurca*) growing in White Park, Riverside, Cal. A number of branches produced in part leaves which were light yellow or almost white in color. The percentage of calcium was found to be considerably greater in the green leaves than in the yellow leaves. The percentage of magnesium was greater in the green leaves, while the percentage of iron and of phosphoric acid was greater in the yellow leaves, indicating that the absence of chlorophyll is not likely to be due to lack of iron or phosphoric acid. The leaf stems of the yellow leaves contain more of each of the elements determined than the leaf stems of the green leaves, which might be interpreted to indicate that the transfer of mineral nutrients did not take place as freely from the leaf stems in the yellow branches as in the green ones.

In the last set of analyses of privet leaves given in Table V, the yellow margins also contained more iron and phosphoric acid than the green midribs, but about one-half as much calcium and a little less magnesium. Of the two elements necessary for chlorophyll formation, iron and magnesium, iron is present in larger amounts in the yellow

leaves than in the green leaves, and the percentage of magnesium is almost as great.

TABLE V.—Analysis of normal green leaves and of yellow leaves of privet. Collected on July 1, 1916

Part analyzed.	Description.	Percentage on dry substance.			
		Fe.	Ca.	Mg.	P ₂ O ₅ .
Entire leaf.....	Normal green leaves (entire leaf).	0.0225	2.03	0.250	0.481
Leaf stems of above.....	do.....	.0108	.844	.131	.326
Yellow leaves.....	Entire leaf yellow, including midrib.	.0388	1.035	.196	1.165
Leaf stems of above.....	do.....	.0386	1.350	.174	.522
Yellow leaf margins.....	Green midrib, yellow leaf spread.	.0312	.806	.221	1.305
Green midribs of above.....	do.....	.0242	1.78	.259	.815
Leaf stems of above.....	do.....	.0197	.985	.148	.448
Average of yellow margins and green midribs.		.0277	1.293	.240	1.060

SUMMARY

Previous studies by this office in southern California have shown that the percentage of mottling of the Citrus leaves varied inversely with the humus content of soils in Citrus groves; that decomposing organic matter increases the amounts of soluble salts in the soil; and that a system of basin mulching in Citrus groves, especially on certain soil types, has produced an improvement in tree growth and fruit setting in comparison with the furrow system of irrigation and surface cultivation.

The purpose of the study here reported was to see if mottled Citrus leaves showed a deficiency of the mineral elements directly affecting chlorophyll formation. If this were the case, better leaf growth on Citrus trees in orchards well supplied with active organic matter might be associated with the greater amount of soluble mineral plant food in a soil well supplied with decomposing organic matter.

It was found that orange and lemon leaves very badly mottled contained higher percentages of iron, calcium, magnesium, and phosphoric acid than healthy leaves, the average percentage of the entire leaf being considered.

The leaves in the medium stages of mottling sometimes contained more and sometimes less of these elements than healthy leaves.

In nearly all cases the midribs of the healthy leaves contained less of the above-mentioned elements than the mesophyll tissue. In badly mottled leaves the midribs contained a higher percentage of calcium

than the mesophyll tissue, usually as much magnesium, and usually more phosphoric acid.

With very few minor exceptions, the leaf stems contained less iron, calcium, and magnesium than either the midrib or mesophyll area in both healthy and mottled leaves. The percentages of calcium, magnesium, and phosphoric acid, however, increased in the leaf stems of badly mottled Citrus leaves, but usually not in the medium mottled leaves.

Old leaves contained higher percentages of calcium and magnesium than new leaves not fully grown.

In all the Citrus leaves analyzed, the phosphoric acid was quite uniformly distributed in the midribs, the mesophyll tissue, and the leaf stems (regardless of age or stage of mottling), indicating that phosphoric acid is early and freely transferred through the conducting tissue to the mesophyll areas.

Sharply outlined yellow spots in the mesophyll areas of orange leaves contained less calcium, magnesium, and phosphoric acid than the green parts (mostly veins) of the leaves.

Green leaves and the green parts of spotted leaves of the golden privet contained about twice as much calcium and appreciably more magnesium than the yellow leaves. Yellow leaves and the yellow parts of spotted leaves contained more iron than and about 2.5 times as much phosphoric acid as the green leaves or green parts of spotted leaves.

Leaf stems of green privet leaves contained lower percentages of iron, calcium, magnesium, and phosphoric acid than the leaves.

Leaf stems of yellow privet leaves contained about one-half as much phosphoric acid as the leaves; the percentages of iron and magnesium were about the same, while the leaf stems contained more calcium than the leaves. The leaf stems of yellow privet leaves contained higher percentages of calcium and magnesium than the leaf stems of green privet leaves.

Judged by a comparison of the average percentages of the inorganic elements determined in healthy Citrus leaves and in leaves in the medium stages of mottling, the data obtained did not show that the initial mottling could be accounted for by deficiency in the transfer of the iron, calcium, magnesium, and phosphoric acid from the conducting system of the leaf stem and midrib to the mesophyll tissue.

On the other hand, sharply localized yellow areas in old orange leaves contained less of these elements than the adjoining green areas (mostly veins), but whether that relation obtained in the initial stage of mottling was not determined.

In very badly mottled Citrus leaves there was in general an increase in the percentage of these elements in the conducting tissues, including the leaf stems, indicating difficulty in their transfer to the mesophyll tissues in very advanced stages of mottling, probably because the leaf had become functionless.

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EFFECTS OF MUSCULAR EXERCISE AND THE HEAT OF THE SUN ON THE BLOOD AND BODY TEMPERATURE OF NORMAL PIGS¹

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INTRODUCTION

This work was carried out, in part, along with our studies of the blood of normal resting pigs. The work first suggested itself on June 25, 1915. We had just started our work on normal adult pig's blood, and had confined in a small outside pen several pigs. These pigs had been quiet, but it was noticed that a certain red sow was forced to lie directly in the sun. This animal was breathing rapidly, but was thought to be normal. On taking her temperature it was found to be 106° F., and the blood clotted at 15 seconds (about 60 seconds being normal). By permitting the animal to lie in the shade the body temperature soon returned to normal, as did the clotting time of the blood. A differential count of the leucocytes at this time showed an unusually high percentage of polymorphs, and an unusually low percentage of lymphocytes. Although we were familiar with the fact that muscular exercise causes these changes in the blood of man, we were surprised to learn that heat could cause similar changes in the blood of the pig. It was therefore decided to continue these studies of the pig.

Some time after this experience several papers appeared in the literature dealing with the effects of muscular exercise upon the blood of man; and, in carefully going over the literature, it was observed that the cause of the various changes in the blood of man resulting from muscular exercise was unsettled. It is therefore hoped that the results of this work on the pig may throw a little light on these phenomena.

METHODS OF STUDY

The animals used in this work were normal hogs weighing about 100 pounds. They had previously been in a resting state for a couple of days. They were taken from their pens, placed in a special hog crate, and the various determinations made. The results of these examinations were added to our data concerning the examination of normal

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² I wish to take this opportunity to express my great appreciation of the valuable assistance rendered by my associate, Mr. Arthur L. Anderson, in carrying out this work.

hog's blood. The animals were then removed from the crate and exercised for a certain length of time, or placed in a small wire pen, exposing them to the sun according to the series to which they belonged. Some pigs were very easy to exercise, and would drive freely for several minutes and would begin to show symptoms of fatigue and a high body temperature 10 to 15 minutes after the exercise had started. Many pigs, on the other hand, were difficult to drive; it is difficult to get them to move at a faster gait than a walk, and with such animals it usually requires about one-half hour of exercise before they show fatigue and an increase in body temperature. Immediately after the exercise or sun exposure the various blood examinations were again determined.

In a number of animals dry spreads were made at certain intervals, covering a period of 24 to 48 hours or longer, following the exercise or exposure to the sun. In this manner the leucocytic curve was established.

The methods of technic were similar to those used in our studies on the blood of normal pigs. The temperature throughout each experiment was recorded.

I.—EFFECTS OF MUSCULAR EXERCISE

A.—EFFECTS ON THE RED CORPUSCLES

It is well established that exercise in man causes an increase in the number of red corpuscles. Tornow¹ found in experiments on soldiers after long marches an increase of about 9 per cent in the number of red corpuscles. Hasselbalch and Heyerdahl (2) in two experiments found an increase of 13 and 17 per cent. Boothby and Berry (2) found an average increase of 19 per cent, but the increase occurred only when sweating was marked. Willebrand (5) found in 12 young men during gymnastic work an increase which varied between 2.9 and 23.4 per cent; and the length of time consumed in returning to normal was quite variable. Zuntz and Schumberg (5, 7), in a study of German soldiers during marches, obtained an average increase of 9 per cent. Hawk (5), in his studies of college athletes, found an increase from 7.3 to 26.7 per cent. Schneider and Havens (7), in their studies on college athletes, found an increase which varied between 3.2 and 22.8 per cent.

In animals Cohnstein and Zuntz (5) examined the blood of rabbits, after subjecting the animals to a systematic harassing for several minutes. They found a slight decrease in the number of red corpuscles and an accompanying leucytosis.

In the blood of 15 pigs studied in this laboratory there were practically no changes in the number of red corpuscles (Table I). There were slight individual variations in the counts before and after exercise; in some cases there was an increase and in others a decrease following the exercise. These variations, however, were no different than those found in

¹ Reference is made by number to "Literature cited," p. 18a.

normal resting pigs (see "Control experiments"). The average count before and after exercise showed 6,098,733 red cells per cubic millimeter before and 6,053,666 after exercise.

TABLE I.—Effects of muscular exercise on 15 normal pigs

BEFORE EXERCISE														Blood.						Differential count.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Animal No.	Weight of animal.	Condition of animal.	Color and sex of animal.	Temperature of animal.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
					Clotting time.	Hemoglobin.	Specific gravity.	Number of erythrocytes.	Number of leucocytes.	Lymphocytes.	Polymorphs.	Mononuclears.	Eosinophiles.	Masts.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
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Several investigators, working with the blood of man, have found a leucocytosis following muscular exercise. Hawk (5) cites three investigations in which a leucocytosis was found after muscular work, the number of leucocytes varying from 11,400 to 22,200 (normal, 8,000 to

B.—EFFECTS ON THE LEUCOCYTES

10,000). Zuntz and Schumberg (5, 7) found an increase of 43 per cent in the number of leucocytes in soldiers, following marches. Hawk, in his college athletes, found an increase of from 21 to 104.4 per cent, with an average of 57.0 per cent. Schneider and Havens (7) found an increase which varied from 13.8 to 130.2 per cent and returned to normal in a very short time (30 to 45 minutes).

In animals Cohnstein and Zuntz (5) found a leucocytosis in rabbits, following muscular exercise. In pigs we have found, on an average, an increase of 18.88 per cent. This increase, however, was not uniform; and in some cases it was lower after exercise than before (for explanation, see "Discussion of results" and "Control experiments").

I.—CHANGES IN DIFFERENTIAL COUNTS

Larrabee (3, 5) not only found a leucocytosis in the blood of the long-distance runners which he examined but he also found the polymorphs to be increased. The eosinophiles were absent in three cases and much reduced in the fourth. The number of transitionals was increased. Zuntz and Schumberg (5, 7) obtained an increase of polymorphs from 6 to 11 per cent, and a decrease in the lymphocytes from 3 to 17 per cent. Schneider and Havens (7) found an increase of 9 to 45 per cent in polymorphs, and a decrease of 14 to 55 per cent in the lymphocytes. They found no definite change in the proportions of the various kinds of leucocytes at the close of the exertion, but slowly thereafter and throughout a period of from 1 to 2 hours the polymorphs increased and the lymphocyte index (including lymphocytes, mononuclears, and transitionals) decreased. They found that the normal proportions returned after about 2 to 4 hours. They state that the changes in the differential count continued long after the normal number of leucocytes had been returned. Burrows (7), on the other hand, in a study of a single case, found that exercise decreased the polymorphs and increased the lymphocytes.

In pigs we have found a condition similar to that reported by Larrabee, Zuntz, and Schumberg and Schneider and Havens for man, except that the normal proportions did not return until after a much longer period. Differential counts of the leucocytes indicate a slow destruction of the lymphocytes of the blood following muscular exercise and an increase of the polymorphs.

In 15 experiments in which the examinations were made shortly after the exercise there was an average decrease of 12.61 per cent of lymphocytes, an average increase of 15.13 per cent of polymorphs, an average decrease of 2.34 per cent of eosinophiles, a very slight increase in the mononuclears, and a slight decrease in the masts.

2.—DIFFERENTIAL CURVE

In five experiments an attempt was made to follow these changes in the differential count until the normal proportions returned. Spreads were made at intervals of a few hours and covering a period of 24 to 48 hours.

(a) LYMPHOCYTE CURVE.—The lymphocytes were decreased, the height of the reaction occurring from two to seven hours after exercise, with an average of four hours. At this time they were decreased 29.43 to 39.77 per cent, the average being 32.75 per cent. After the height of the reaction there was a gradual return to the normal proportions, which took place after 36 to 48 hours.

(b) POLYMORPHONUCLEAR CURVE.—The polymorphs were increased, the height of the reaction occurring from two to seven hours after exercise, and the average high point of the reaction was four hours after exercise. At this time they were increased 33.79 to 44.77 per cent, with an average increase of 39.88 per cent for the entire five animals. Following the height of the reaction the normal proportions gradually returned, and the time required varied between 36 and 48 hours.

(c) EOSINOPHILE CURVE.—The eosinophile curve varied from that found in the lymphocytes and polymorphs in that these cells showed a double curve. Shortly after the exercise (4.5 hours) the eosinophiles dropped to zero, or nearly zero; following this there was a gradual increase until these cells became much higher than the percentage normally found. The height of this latter curve was reached about 24 hours after exercise. Following this, the normal proportion gradually returned, and the count was again normal at 30 hours or longer.

Table II details the changes in the eosinophile count in five experiments.

TABLE II.—Changes in the percentages of eosinophiles covering a period of 48 hours following the muscular exercise of pigs

Animal No.	Normal percent- age.	Lowest percent- age.	Difference.	Hours after exercise when percent- age is lowest.	Highest percent- age.	Hours after exercise when percent- age is highest.	Hours after exercise when return to normal occurs.
			<i>Per cent.</i>				
1.....	0.56	0.00	0.56	4.5	11.01	24	Not established.
2.....	3.00	.58	3.42	4.5	9.68	24	Do.
3.....	1.37	.00	1.37	4.5	11.88	24	Do.
4.....	13.11	.17	12.94	7.0	15.63	24	Do.
5.....	4.82	.00	4.82	2.0	11.02	29	About 30 hours.

(d) MAST CURVE.—The behavior of the mast cells was very similar to that of the eosinophiles, and the curve was irregularly parallel with the eosinophile curve.

(e) MONONUCLEAR CURVE.—The mononuclears were decreased at first, and later returned to normal. Their behavior was quite similar to the lymphocytes. Figure 1 shows in detail the various curves for one animal following muscular exercise.

C.—EFFECTS ON HEMOGLOBIN

Boothby and Berry (2) found an increase in the percentage of hemoglobin, and this increase occurred only when the red corpuscles were

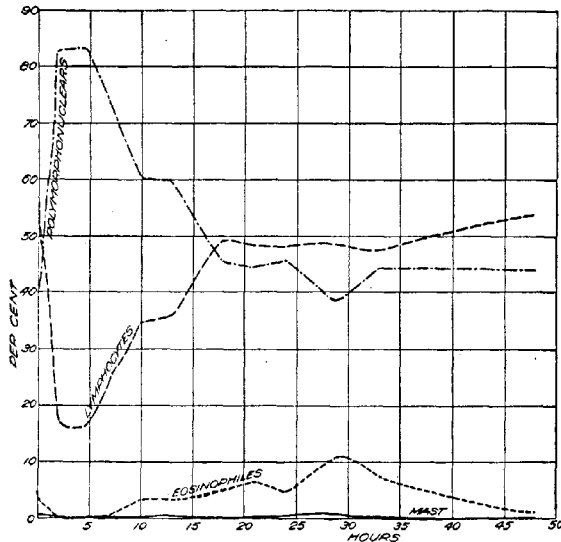


FIG. 1.—Graphs showing the detail of leucocytic changes in the blood of one pig following muscular exercise.

increased. These changes did not occur unless there was marked perspiration. These workers found the increase to vary between 7 and 11 per cent. Schneider and Havens (7) found the changes in hemoglobin, on the whole, proportionate, but not always equal, to the increase in the number of red corpuscles. They found the increase to vary between 3.5 and 10.9 per cent.

In the pig we have found an average increase of 3.13 per cent after exercise. The increase was not consistent. The percentage was found to be higher in 8 cases, lower in 4, and even in 3.

D.—EFFECTS ON SPECIFIC GRAVITY

The specific gravity of the blood of man in muscular activity has been studied by Jones (6). He found that it usually varied directly with the red corpuscles. He reports, however, that gentle exercise is accompanied by a fall, while the more prolonged or violent forms of exercises are accompanied by a rise in the specific gravity. Zuntz and Schumberg (5, 7) constantly obtained an increase in the specific gravity of the blood. Schneider and Havens (7) found that exercise invariably caused a rise.

In pigs we did not find an increase in specific gravity when the entire experiment was considered.

E.—EFFECTS ON CLOTTING TIME

As one would naturally expect, the clotting time was greatly increased. The clotting time seems to be varied in a rough way with the body temperature of the pig.

CONTROL EXPERIMENTS

Several control experiments were carried out, whereby blood counts were made on normal resting pigs at various intervals. In making the wet counts the same pipette was used in each experiment, and the blood samples were obtained from a fresh vein in either ear. The dry spreads were obtained in a similar manner—that is, a fresh clean vein was used in making each spread.

Results of this work showed that, although in some pigs the results are quite uniform both in the wet and dry counts, it is often found that the blood of the pig is subject to considerable variation at different times.

In some pigs the wet counts were very uniform and showed no variations other than normal. In others there was sometimes as much as 1,000,000 red corpuscles per cubic millimeter difference in two examinations on the same animal. The differential count of leucocytes also showed similar results. In some pigs the differential count covering a period of 24 hours was quite uniform and showed very slight changes, varying between zero and 2 to 3 per cent. In others, however, there was marked variation. The individual leucocytes varied at different times, and the greatest variation occurred in the polymorphs and lymphocytes. Five experiments dealing with the differential counts were made in normal resting pigs and covering a period of 24 hours (12 counts made).

II.—EFFECTS OF HEAT OF SUN

In this series of five experiments on the effects of the sun's heat only the changes in the leucocytes were studied. The differential curve for a period of 36 to 72 hours was worked out. The results in general were quite similar to those obtained in the animals which were given muscular exercise.

(a) LYMPHOCYTE CURVE.—The lymphocytes were decreased in number, the height of the reduction occurred from 4.5 to 7 hours after being placed in the sun, and the average for the five experiments was 6 hours. At this time they were decreased 5.06 to 17.01 per cent, the average being 11.48 per cent. After this there was a gradual return to the normal proportions, which took place after 15 to 48 hours, the average being 29 hours. The greatest change required the longest time to return to normal.

(b) POLYMORPHONUCLEAR CURVE.—The polymorphs were increased in numbers, the height of the reaction occurred from 4.5 to 7 hours after being placed in the sun, and the average for five experiments was 6 hours. At this time they were increased 3.79 to 21.63 per cent above the normal, the average increase being 11.4 per cent. After the height of the reaction there was an irregular return to the normal, which took place between 15 and 72 hours or more. The lowest change required the least time to return to normal.

(c) MONONUCLEAR CURVE.—There was a slight decrease in the number of mononuclears. The height of the reaction occurred about 6 hours after being placed in the sun. Following this there was a gradual increase to the normal proportions. This increase was quite irregular. In general, the behavior of these cells was quite similar to the lymphocytes.

(d) EOSINOPHILE CURVE.—The behavior of the eosinophiles was, in general, very similar to this class of cells following muscular exercise. If the normal percentage of these cells was rather high, there was always a decrease, the height of the reaction occurring about 5 to 7 hours after being placed in the sun. Following this there was a gradual increase in the number of these cells, the increase running far beyond the normal percentage. The height of this increase was attained from 13 to 27 hours after being placed in the sun. Then there was a gradual return to the normal percentage. If the normal percentage was low, the first decrease did not occur, but there was a gradual increase until about 13 to 27 hours after being placed in the sun, when the height of the reaction occurred. After this there was a gradual return to the normal proportions.

TABLE IV.—Details of the changes in the percentages of eosinophiles covering a period of 72 hours following exposure of pigs to the sun

Animal No.	Normal percentage.	Lowest percentage.	Difference.	Hours after exposure in sun when percentage is lowest.	Highest increase in percentage.	Hours after exposure in sun when increase in percentage is highest.	Hours after exposure in sun when return to normal occurs.
1.....	0.79	Gradual increase.	Gradual increase.	5.00	13	Not established.
2.....	4.95	0.36	4.59	5	9.10	31	60 hours.
3.....	1.37	Gradual increase.	Gradual increase.	5.12	27	Not established.
4.....	1.57	do.	do.	4.08	27	48 hours.
5.....	2.14	.19	1.95	7	7.88	27	60 hours.

(e) Table IV shows in detail the changes in the percentages of eosinophiles at the turning points of the curve.

MAST CURVE.—The behavior of the mast cells was very similar to that of the eosinophiles, and the curve was irregularly parallel with the eosinophile curve.

Figure 2 shows in detail the various curves for one animal following exposure to the sun.

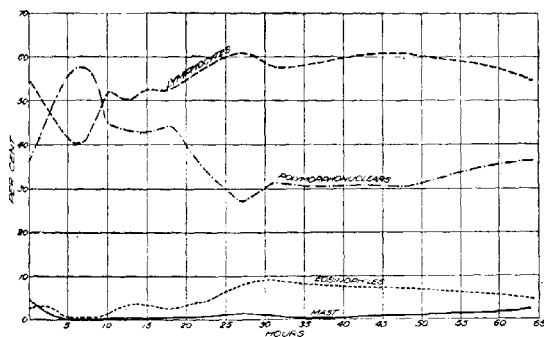


FIG. 2.—Graphs showing the detail of leucocytic changes in the blood of one pig following sun exposure.

III.—EFFECTS OF MUSCULAR EXERCISE AND HEAT OF THE SUN ON BODY TEMPERATURE

The body temperature of animals is known to show more variations than the body temperature of man. In animals many external conditions cause variations in body temperatures. Lee (1) has called attention to recent findings of the New York Commission on Ventilation with respect

to an apparent relationship between the body temperature of man and the temperature of his environment, even under ordinary conditions of living. It was found, for example, that during summer months the rectal temperature at 8 a. m. of people living in their own homes was conditioned by the average atmospheric temperature of the preceding night. If the temperature had been warm, the body temperature in the morning was high; if cool, the body temperature was low. The variation was about 1° F. for 20 degrees of atmospheric temperature. The body temperature was lowered by confinement in an atmosphere of 68° and 50 per cent relative humidity and raised by confinement at 75° with the same humidity, or still more by 86° with 80 per cent humidity. The actual body temperatures found at these stages, respectively, were 98°, 98.5°, and 99.3°.

In extreme atmospheric conditions greater elevations of temperature are known to occur (1). A stay of about three hours in an atmosphere averaging 104.7° in temperature and 95 per cent relative humidity may produce a rise of several degrees in the body temperature of a normal adult man.

Schroeder and Mohler (8), in their bulletin dealing with the tuberculin test of hogs, state that, when the temperature of a number of hogs is compared, the difference found is of such magnitude that they are at a loss to conclude what should be regarded as normal. Other than stating that the normal variations that occur in individual hogs are very great, they do not give any figures. These workers have also found that fat hogs have a higher temperature than lean ones and that a higher temperature induced by exercise or some other temporary cause persists longer in fat than in lean hogs.

We have not collected any large amount of data concerning temperature variations in normal pigs other than the temperature records which accompanied our blood experiments. These experiments, however, very clearly demonstrated the extreme variability in the temperature of pigs.

Merely being in the direct rays of the sun will cause a marked increase in the body temperature, the increase, of course, varying with the atmospheric temperature and relative humidity. On a hot day a normal pig exposed to the direct rays of the sun may show a temperature as high as 106° to 108° F.

The handling of or working among a group of pigs for only a few minutes can cause a marked increase in their body temperatures. For example, a black sow weighing 102 pounds had been quiet in a small pen for two days. Her rectal temperature was then 102.8°. She was then picked up and placed in a hog crate, the operation requiring only a few minutes, but it caused a rise in the rectal temperature to 104°.

A black sow weighing 130 pounds and in good condition, but not fat, had been confined for several days in a small pen. Before taking her out

of the pen her temperature was 102.3° . She was driven slowly across a 50-foot lot and placed in a crate, her temperature at this time being 103.5° . She was in the crate for 30 minutes, and during this time fought some. At the end of this period her temperature was 105.8° . She was then exercised for 15 minutes at a slow pace (could not get her to go much faster than a walk), and following this exercise her temperature was 107.8° . She was then placed in the crate again, the various blood examinations were made, and just before taking her out of the crate she showed a temperature of 108.1° and was dyspneic.

The mere handling of the animals, as holding for examination or placing them in a crate, especially if they offer any resistance, will cause an increase in the temperature of a degree or two, even when the temperature is already high. For example, a black male weighing 105 pounds showed a pen temperature of 102.4° . After placing him in the crate the temperature was increased to 104° . The animal was still just before removing him from the crate, and the temperature had been lowered to 103° . The animal was then exercised for 15 minutes (on a rather cool day), and after the exercise the temperature was increased to 105.6° . After placing the animal in the crate, the temperature was increased to 106.4° , and just before removing from the crate it had been lowered to 106.2° . Very slight struggling in the crate will keep an already high temperature elevated and tend to increase a low one.

Although it was not our intention to see how high the temperature would go, it might, however, be worthy of recording that the highest temperature after exercise was noted in a red sow weighing 108 pounds. The animal was in very good condition. Before exercise the sow showed a temperature of 102.4° . After exercising for one-half hour at a slow gait, the temperature was increased to 109.4° .

After exercising and obtaining the blood samples, if the changes were not to be followed further, the animals were given a cold shower bath, which quickly lowered the temperature and which the animals thoroughly enjoyed.

If allowed to remain quiet, the temperatures would return to normal in one-half to one hour.

DISCUSSION OF RESULTS

CHANGES IN THE ERYTHROCYTES AFTER MUSCULAR EXERCISE

Muscular exercise does not cause an increase in the number of red corpuscles in the blood of the pig. This is difficult to explain. It is possible that there was an increase following the exercise; but the normal proportions had returned before the count was made, although such an explanation seems hardly plausible in light of the work of Zuntz and Schunberg (5, 7), who found an increase in the number of red corpuscles in soldiers after long marches. Schneider and Havens (7) found the height of the erythrocyte curve was not reached until 75

minutes after an 0.8 mile run, and the normal proportions did not return for 2 hours. In pigs our counts were made in all cases quite within this time.

Hawk (5) advances six possible explanations for the increase of erythrocytes in exercise. These are (1) the production of new corpuscles; (2) concentration of the blood through increased urine formation and copious sweating; (3) concentration of the blood through increased evaporation in the lungs; (4) concentration of the blood through vaso-motor contraction and rise in blood pressure; (5) sudden passage into the circulating blood of a large number of cells lying in various parts of the body and inactive before the time of muscular exercise; and (6) passage of fluid from the blood to active muscles.

Hawk concludes that the number of red corpuscles produced by muscular exertion is due primarily to the passage into the circulating blood of a large number of cells lying in various parts of the body and inactive before the time of the muscular exercise.

We can hardly see how this is possible in the case of the red corpuscles which remain in a closed, constantly circulating system. Further, if this conclusion is correct, we would expect an increase in the number of erythrocytes in the pig.

Schneider and Havens (7) conclude that the increase in erythrocytes is due to a concentration in the peripheral capillaries. Willebrand (5) believes that the withdrawal of water from the blood by the working muscles is the primary cause of concentration. Zuntz and Schumberg (5, 7) accept Willebrand's explanation.

If either of these conclusions is correct, we would expect the blood of the pig to show an increase in the erythrocytes following exercise. Tornow (2) concluded that the increase in the red corpuscles corresponded roughly to the increased density of the blood as a result of sweat caused by muscular work. Similar conclusions can be drawn from the work of Hasselbalch and Heyerdahl (2), since there was no definite reaction after the first run, while after the second run there was a very distinct rise in the number of red corpuscles. This has been explained by Boothby and Berry (2) on the ground that there was no distinct change in the relative number of red corpuscles until sufficient time had elapsed for an appreciable amount of sweating to have occurred.

Boothby and Berry conclude from their studies that the increase in the percentage of hemoglobin and red corpuscles occurs under conditions of work causing an appreciable amount of perspiration. If no perspiration occurs, there is no such increase.

Evidence in the pig rather tends to confirm this theory, since the pig is an animal which does not sweat and does not show any increase in the number of red corpuscles under various degrees of muscular exercise. The same has been found to occur in the case of the rabbit, another animal which does not sweat.

LEUCOCYTES AFTER MUSCULAR EXERCISE

An increase in the number of leucocytes occurs in the pig. This increase was slight, compared to the increase found in man.

Schneider and Havens (7) found that the normal proportion of leucocytes usually returned about 30 to 45 minutes after the exercise period. It is quite possible that in the pig a similar phenomena occurred and that the normal proportions had nearly returned by the time we made our after-exercise counts. This fact needs further investigation.

To account for the leucocytosis, Hawk (5) concludes that it is due to an accumulation of leucocytes in the peripheral circulation. Zuntz and Schumberg (5, 7) believe that, since the white corpuscles increase so much more than the red, a different explanation must obtain for their increase. They hold that the passing of wandering cells from the tissues into the general circulation is an adequate explanation. Schneider and Havens conclude that concentration of blood in the peripheral capillaries is the chief cause of the increase in the number of leucocytes. They also state that the contraction of the voluntary muscles accelerates the flow of lymph, throwing lymph rich in leucocytes into the blood.

Our results in the pig show that the influences which caused an increase in the number of red corpuscles in the blood of man can not be used to explain the increase in the number of leucocytes. It would seem that the explanation proposed by other workers—namely, that leucocytosis results from the passage of leucocytes from the tissues and lymphatic system into the general circulation as a result of muscular contraction—is quite satisfactory.

ADRENALIN THEORY

Schneider and Havens (7) think that adrenalin is primarily responsible for the changes in the number of blood corpuscles in the peripheral circulation. They state that during muscular inaction a large mass of the blood is directed to the splanchnic area, where it probably stagnates and gives up plasma as lymph. There is also throughout the remainder of the body, especially in the limbs, an accumulation of lymph. With the onset of muscular activity the carbon-dioxid content of the blood rises, this carbon dioxide stimulates the central nervous centers which regulate the secretion of the suprarenal glands, hence, the output of adrenalin is increased. The adrenalin causes a constriction of the blood vessels of the splanchnic area; this forces the stagnant red corpuscles into the general circulation, thus giving the rise in specific gravity, hemoglobin, erythrocyte, and leucocyte content of the peripheral blood. The increase in red corpuscles and hemoglobin makes it possible to supply more readily the greater demand for oxygen made by the active muscles. Shortly after the close of the exercise the carbon-dioxid content of the blood falls below normal. As a result the discharge of adrenalin becomes subnormal and the blood once more accumulates in the splanchnic area, so that there is a gradual return to the normal composition and even a temporary subnormal content in red corpuscles.

This is a beautiful explanation and the work of Schneider and Havens tends to prove this theory. If adrenalin is the primary factor concerned in increasing the number of red corpuscles, hemoglobin, etc., we fail to understand why an increase in these factors did not result in the pig.

CHANGES IN THE DIFFERENTIAL COUNT

The changes in the percentage of the different kinds of leucocytes can be explained on the theory of rapid aging of the leucocytes due to increased wear. Cells grow old under physiological conditions; it is difficult to follow all of the various stages, because every transition form is not available for presentation, because some of the stages are passed over too quickly, or because certain stages in the life history of different cell types may be very similar to each other. Transitional forms may be met with which can not be named, because nomenclature itself is incomplete.

The signs of old age in a cell are (4, p. 18): (1) The cell body becomes relatively larger; (2) the nucleus becomes spherical and relatively smaller; and (3) the nucleus becomes indented and polymorphous.

A cell with a round single nucleus is younger than a cell with a polymorphous nucleus. In the blood of the pig, following muscular exercise and exposure to the sun's heat, we find a decrease in the mononuclear elements and an increase in the polymorphonuclear elements, showing that the cells are becoming old faster than new cells are being produced or that the rate of aging in a cell has been increased. The mast cells are increased in numbers and the majority of these cells resemble lymphocytes which contain many dark granules. Gruner (4, p. 19) states that the lymphocyte may develop mast-cell granules when suitable conditions arise. Thus, we find a few of the lymphocytes which assume the form of mast cells and may be classed under this heading.

Muscular exercise and increased body temperature both very likely play an important part in hurrying the life cycle in the leucocytes.

VARIATIONS IN BODY TEMPERATURE

It is quite evident that the heat-regulating mechanism in the hog is a very poor one. Schroeder and Mohler (8) call attention to the fact that the hog is an animal that is ordinarily incased in a thick layer of fat, which is a poor conductor of heat and in which the circulation of blood is very meager. Over the fat a skin is stretched in which the circulation of blood is relatively small; and this skin, unlike that of a man or a horse, does not take a prominent part in regulating the body temperature through the agency of radiation and perspiration. The covering of a hog may be regarded as an excellent means for preventing the escape of heat from the body rather than for regulating the temperature of the body; hence, we have conditions that probably permit a more rapid production than escape of heat. These workers also state that when the temperature of a number of hogs is compared the difference found is of

such magnitude that they were at a loss to conclude what should be regarded as normal.

There are several factors which can possibly account for the wide variations found in the temperature of normal hogs: (1) The condition of the animal—that is, the amount of fat; (2) the temperature of the atmosphere and the percentage of humidity. A fat hog would have a much higher body temperature on a hot humid day than a lean hog, and even on a cool day a slight variation may occur. Schroeder and Mohler conclude that fat hogs have a higher temperature than lean ones, and that a higher temperature induced by exercise or some other temporary cause persists longer in fat than lean hogs.

Since environment can cause a marked variation in the temperature of man, with his excellent heat-regulating mechanism, we would expect that similar conditions would cause a greater variation in the case of the pig with a poor heat-regulating mechanism.

SUMMARY

(1) Blood examinations in normal resting pigs, covering a period of 24 hours, may be quite uniform; but in some animals there is marked variation throughout the period.

(2) Observations made upon a number of animals leads to the conclusion that muscular exercise does not cause an increase of red corpuscles in the peripheral circulation of the pig.

(3) Results based on only one or a few experiments may lead to wrong conclusions, owing to the variability in the blood of pigs.

(4) Evidence given by work with the pig tends to confirm the theory of perspiration being responsible for the increase in the number of red corpuscles following muscular exercise in man.

(5) Muscular exercise in the pig is usually followed by a leucocytosis.

(6) This leucocytosis is probably the result of muscular exercise forcing leucocytes into the general circulation from the tissues.

(7) Muscular exercise leads to marked changes in the differential counts. The mononuclear elements are decreased, and the polymorphonuclear elements are increased. The height of the curve is reached several hours after exercise, and the normal proportions do not return for many hours.

(8) Exposure to the sun causes similar changes in the differential curve.

(9) These changes under both conditions are the result of increased rate of aging of the leucocytes, the cells becoming older faster than young cells are being produced.

(10) Muscular exercise and heat of the sun lead to a marked increase in body temperature.

(11) Body temperature changes are more pronounced in fat pigs than lean ones, but even in pigs weighing 75 to 100 pounds marked changes are likely to occur.

(12) Increased atmospheric temperature and increased percentage of humidity lead to increased body temperature.

(13) Blood examinations of pigs which are to be used for clinical records should be taken from animals which have been confined in a small cool pen for at least 24 hours, and better, 48 hours. The animals must be kept absolutely quiet and not worried. Feeding and watering should be regular. The daily blood examinations should be made at the same time on each day.

(14) Temperature records which are to be used for clinical records should be taken from pigs kept in a cool, shady pen. The animals should not be exercised or worried when the temperatures are taken. If the animals are chased around the pen in endeavoring to obtain the temperature, the last temperatures taken may show a marked rise. For tuberculin work where the temperatures are used it would be best to keep them confined in a crate throughout the test.

(15) The condition of the animal (amount of fat), the temperature of the atmosphere, and the percentage of humidity are factors which should be considered in determining the normal temperature of the pig.

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